

## Lentiviral vectors titration using real-time PCR

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### ABSTRACT

Lentiviral vectors (LVs) are useful vehicle for genetransfer to dividing and non-dividing cells and genetic manipulations. However, the use of lentiviruses in studies requires an accurate titration technique. Quantitative real-time PCR (qPCR) is a sensitive technique for the indication and quantitation of retrovirals particles. In this study, we used the qPCR for lentiviral vector titration. The puromycin resistance gene as templates for an SYBR green-based real-time qPCR method and detect lentiviral copy number integrated lentiviral DNA. Consequently, this study showed that the using of antibiotic resistance genes viral particles titration may be efficient with highly accuracy.

Keywords Lentivirus; qPCR; Titration methods; Puromycin.

### INTRODUCTION

Lentiviral vectors (LVs) are useful vehicle for genetransfer agent to dividing and non-dividing cells. Lentiviruses were produced by transfected 293T packaging cells with plasmids containing lentiviral vectors components. Lentiviral transduction efficiencies of up to 95%, with low levels of cell toxicity in transduced cells[1]. However, in order to perform transgenic manipulating, we need methods to analyze the titration of lentiviral vectors.

Various methods for titration have been reported[2, 3], including p24 antigen ELISA (enzyme-linked immune sorbent assay), RNA titers, reverse transcriptase (RT) activity, fluorescence activated cell sorting (FACS), and quantitative polymerase chain reaction modifications (qPCR). However, some of these techniques aren't appropriate for estimating the numbers of viral particles. For example, nonfunctional and functional particles measure by p24 antigen ELISA, RNA titration and RT-assay[4, 5]. The common and simple technique to quantify functional vector titers utilizes eGFP fluorescence and fluorescence-activated cell sorting (FACS)[6]. However, FACS analysis of transgene expression is limited to fluorescent

proteins and may not recognize cells that containing multiple copies of transgenes. The most precise and difficult titrating method is detection of vector DNA integration in transduced cells[7]. Usually, the copy number of a functional vector is the number of vector particles required to transduce a single cell in a defined volume, and the number of integrated DNA lentiviral copies per cell by estimate by real-time PCR assay[8]. Antibiotic resistance gene as selection marker presents in all generation of LV and can be used for detection copy number of virus particles.

In this study, we described a quantitative quantitative PCR (qPCR) by using primer sequences that are specific for the puromycin resistance gene to measure the copy numbers of lenti viruses that integrated into the genome after production and transduction.

### MATERIALS AND METHODS

#### Lentiviral vector

EX-M0942-Lv105 plasmid was purchased from (Genecopoeia, USA). PsPAX2 and pMD2.G vectors for viral packaging were purchased from (Invitrogen, USA). These vectors were transformed in DH5α. Plasmids